ABRATES

Journal of Seed Science

ISSN 2317-1545
www.abrates.org.br/revista

ARTICLE

Journal of Seed Science, v.46, e202446019, 2024

http://dx.doi.org/10.1590/2317-1545v46280082

Effect of salicylic acid induction on germination, radicle length, and protein content in chickpea seedlings

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ABSTRACT: Germination is a process of high metabolic activity in plants that involves the use of storage components present in seeds for seedling development. Chemical induction is a process in which different types of compounds are used to promote the activation of secondary metabolic pathways. The objective of the present study was to determine the effect of chemical induction with salicylic acid (SA) (1 and 5 mM) on chickpea seed germination, germination percentage, radicle length and protein content in seedlings. Soluble protein fractions and trypsin inhibitory activity were quantified in the seedlings, and protein patterns were identified by polyacrylamide gel electrophoresis. Treatment with 5 mM salicylic acid decreased both the germination percentage and the length of the radicle. The albumin fraction content of the seedlings was reduced with 1 mM SA. The trypsin inhibitory activity in the globulin fraction decreased in seedlings treated with both SA treatments. Protein electrophoretic patterns from SA-induced seedlings remained similar to those without induction. In a dose-dependent manner, chickpea seedlings exhibited changes in seedling development, concentration of protein fractions, and reduced trypsin inhibitory activity.

Index terms: Chemical elicitation, seed storage proteins, germination.

RESUMO: A germinação é um processo de alta atividade metabólica nas plantas envolvendo a utilização de componentes de armazenamento presentes na semente para o desenvolvimento das plântulas. A indução química é um processo no qual diferentes tipos de compostos são utilizados para promover a ativação de vias metabólicas secundárias. O objetivo foi identificar o efeito da indução química com ácido salicílico (SA) (1 e 5 mM) na germinação das sementes de grão-de-bico, na porcentagem de germinação, no comprimento da radícula e no teor de proteína das plântulas. As frações proteicas solúveis e a atividade inibitória da tripsina foram quantificadas nas plântulas, e os padrões proteicos foram identificados por eletroforese em gel de poliacrilamida. O tratamento com ácido salicílico 5 mM diminuiu tanto a porcentagem de germinação quanto o comprimento da radícula. O teor da fração albumina das plântulas foi reduzido com 1 mM de SA. A atividade inibitória da tripsina na fração globulina diminuiu nas plântulas submetidas a ambos tratamentos SA. Os padrões eletroforéticos de proteínas das partes aéreas induzidas por SA permaneceram semelhantes àqueles sem indução. A parte aérea de grão de bico induziu, de maneira dose-dependente, alterações no desenvolvimento das plântulas, na concentração das frações proteicas e reduziram a atividade inibitória da tripsina.

Termos de indexação: elicitação química, proteínas de armazenamento de sementes, germinação.

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Received: 11/02/2023.
Accepted: 06/04/2024.

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INTRODUCTION

Chickpea is an important legume worldwide at the nutritional level. Two main types of chickpeas are known: the 'Kabuli' and 'Desi' types. Seeds of the 'Kabuli' type is larger in size, lighter in color, and contain approximately 24% crude protein and 6.5% crude fiber on a dry-weight basis. The 'Desi' types have a smaller, rough skinned seed and dark tones, and they have 22.76% and 9.94% protein and fiber, respectively (Rachwa-Rosiak et al., 2015; Singh et al., 2021; Xiao et al., 2022). In addition, chickpea seeds have a high content of branched-chain amino acids (BCAAs) such as leucine, isoleucine, and valine and a low content of sulfur-containing amino acids like cysteine and methionine. The majority of its proteins are albumin and globulin (approximately 75%), and to a lesser extent prolamins and glutelins (Rachwa-Rosiak et al., 2015; Serrano-Sandoval et al., 2019; Xiao et al., 2022).

In addition, chickpea seeds also contain antinutritional elements that can affect their nutritional quality. These elements can be toxic or can affect the absorption of nutrients. Chickpea contains protease inhibitors that interfere with the digestion and assimilation of proteins (Mohan et al., 2016). Several processes are applied to grain legumes to reduce or eliminate the content of antinutritional compounds. Boiling, autoclaving, or microwaving methods reduce the activity of trypsin inhibitors in chickpea by 80-83% (Alajaji and El-Adawy, 2006; Avilés-Gaxiola et al., 2018; Kaur and Prasad, 2021). Additionally, seed germination has been shown to decrease the activity of protease inhibitors (Grosse-Holz and van der Hoorn, 2016; Escandón et al., 2022).

Germination, from a physiological view, is the process that begins with seed imbibition until the embryo grows to emerge through the surrounding tissue structures (Nonogaki, 2019). During seed germination, changes in the amino acid content of different legumes have been observed since this process involves hydrolysis, synthesis and rearrangement of storage components (Rodríguez et al., 2008; Ferreira et al., 2019; Yu et al., 2020). During chickpea germination, small changes in the protein nitrogen proportion, changes in protein profiles, and a reduction in trypsin inhibitory activity occur (Bewley et al., 2013; Marcos-Filho, 2016; Avezum et al., 2022; Sofi et al., 2023).

Salicylic acid (SA) is a phytohormone that plays a crucial role in various physiological processes of plants, including seed germination, helping to regulate hormonal balance, stress response, and seed metabolism. Its application can improve germination rates and seedling health, although its effectiveness varies depending on specific conditions and plant species (Yang et al., 2023). The application of SA at low concentrations (µM order) promotes plant development (Hayat et al., 2010). However, by increasing the applied concentrations (mM order) plant development is delayed (Rivas-San Vicente and Plascencia, 2011; Anaya et al., 2018). The accumulation of endogenous SA is regulated by a family of methyl esterase (MES) enzymes (SA methyl esterases) that are responsible for converting Me-SA (inactive form) to SA (active form) (Gao et al., 2021). SA acts as a signaling molecule, triggering the production of reactive oxygen species (ROS) (Ding and Ding, 2020; Ali, 2021), and these compounds can interfere with seedling development (Gao et al., 2021).

On the other hand, when SA is applied exogenously, the activation of secondary metabolic pathways is induced, promoting the synthesis of nutraceutical compounds such as phytosterols and isoflavones (Baenas et al., 2014; Gao et al., 2015; Świeca, 2015) and the degradation of antinutritional compounds such as protease inhibitors, lectins, and phytates. It has also been observed that the application of SA (mM order) during germination improves the nutritional content of seeds and seedlings (Mendoza-Sánchez et al., 2016; Benincasa et al., 2019).

It is assumed that the application of SA at higher concentrations than those that are synthesized endogenously has effects on the germination and development of the seedlings. These effects on the metabolic state are produced by the hydrolysis reactions of storage components necessary for seedling development, such as the degradation of seed storage proteins, in addition to the activation of secondary metabolic pathways mediated by SA. Therefore, the objective of this study was to determine the effect of exogenous induction of salicylic acid during chickpea germination on the percentage of germination, root elongation, storage protein concentration, electrophoretic patterns, and protease inhibitor content.

MATERIAL AND METHODS

The chickpea (*Cicer arietinum* L.) 'Desi' type cultivar 'El Patrón' was used, which was obtained from the National Institute of Forestry, Agricultural and Livestock Research (*INIFAP Campo Experimental Bajío*, Mexico) harvested in the 2019 season; the chickpea seed was stored in a cold chamber at 10 °C and 50% relative humidity until use.

Seed germination and induction with salicylic acid: chickpea seeds were disinfected with a solution of 1% (v/v) sodium hypochlorite for 10 min, rinsed in distilled water to remove chlorine and soaked in distilled water for 1 h to soften seeds and promote uniform water uptake (Mendoza-Sánchez et al., 2016). Two chemical induction treatments (1 and 5 mM salicylic acid) and a control with distilled water were applied, totaling three treatments. For each treatment, three replications with 100 seeds each. The seeds were placed on top of a layer of blotting filter paper inside a trays and other layer of filter paper was placed on top of the seeds. The seeds were sprayed daily with 30 mL of 1 or 5 mM SA solution or 30 mL of distilled water for the control treatment (Mendoza-Sánchez et al., 2016). The samples were placed in a germination chamber (Seedburo, USA), subsequently closed and kept at 25 °C in the dark. The germination was calculated daily (each 24 h, up to 96 h) by measuring the percentage of radicle protrusion. In addition, the radicle length in each sample was measured daily and up to 96 h (the time of appearance of the first true leaf). With the daily germination number and the radicle length data, the slope of each variable was calculated to determine the germination speed and radicle growth speed (Godínez-Garrido et al., 2021). Subsequently, the seedlings were dehydrated at 60 °C for 24 h (Ayala-Rodríguez et al., 2022).

Preparation of seed and seedling flours: the previously dehydrated seedlings were ground and passed through a sieve (mesh number 60) to obtain the flour. The flour was then stored at -20 °C in a freezer until use.

Extraction of protein fractions: the protein fractions were obtained by sequential solubilization with some modifications (Aguirre-Mancilla et al., 2020). Briefly, for the extraction of the albumin fraction, 1 mL of distilled water was added to 0.1 g of flour. The mixture was vortexed for 15 min and later centrifuged at 16200×g for 10 min to recover the first supernatant (albumin fraction). After that, 1 mL of 0.5 M NaCl, 0.05 M Tris-HCl, pH 8, was added to the flour recovered from the first extraction as a precipitate, and the new mixture was vortexed for 15 min and then centrifuged at 16200×g for 10 min to recover the second supernatant (the globulin fraction).

One milliliter of 55% (v/v) isopropyl alcohol was added to the flour recovered from the second extraction to obtain the third supernatant (prolamin fraction) by means of the procedure indicated above. Finally, 1 mL of 0.01 M borate and 0.5% SDS (w/v), pH 9.3, was added to the flour precipitated from the last extraction to obtain the last supernatant (glutelin fraction) via the same procedure described above. All the protein fraction solutions obtained (albumin, globulin, prolamin and glutelin) were stored at 4 °C in a refrigerator until further use.

Protein quantification: protein from each fraction obtained was quantified by the Bradford method, bovine serum albumin (BSA) was used as a standard in a calibration curve, and absorbance readings were made at 595 nm in a spectrophotometer (Bradford, 1976).

Trypsin inhibitory activity: trypsin inhibitory activity was determined using 15 µL of each protein fraction (PF), which was incubated with 10 µL of trypsin solution (TS) (0.05 mg/mL) and 140 µL of 0.1 M Tris-HCl buffer (pH 8) for 15 min at 37 °C. Subsequently, 20 µL of Nα-benzoyl-DL-arginine 4-nitroanilide hydrochloride (BApNA) was added to the mixture as a substrate and incubated for 30 min. Finally, the absorbance at 405 nm was measured with a spectrophotometer (Erlanger et al., 1961).

The inhibition units (IU/mL) were calculated with the following equation:

$$UI/mL = (AbsTS - AbsTS - FP)/(0.01 \times Vol(mL)\ PF)$$

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE): for SDS–PAGE, a 10% (w/v) polyacrylamide separating gel and a 4% (w/v) polyacrylamide concentrating gel were used. Protein fraction samples were dissolved in sample buffer (0.05 M Tris, 4% SDS (w/v), 2% β-mercaptoethanol (v/v), 12% glycerol (v/v), 0.01% Coomassie blue (w/v), pH 6.8) and loaded with 20 µg of protein per lane. The gels were run at 50 V for 30 min and then at 100 V for 120 min

on a Mini PROTEAN Tetra Cell Chamber (Bio-Rad, USA). After that, the gels were stained with a 0.025% Coomassie blue solution in 10% acetic acid (Schägger and von Jagow, 1987) and photographed on a Gel Doc EZ Imager (Bio-Rad, USA) with Image Lab 5.1 software. The intensity of the protein bands in the polyacrylamide gels was analyzed using ImageJ software (Schneider et al., 2012) (ver. 1.54). The densitometric values of the protein bands were obtained from the average of two measurements from two gels and statistically analyzed (completely randomized) for differences in intensity.

Statistical analysis: a bifactorial analysis with Factor A (time) composed by 4 levels (day 1: 24 h, day 2: 48 h, day 3: 72 h, and day 4: 96 h), and Factor B (salicylic acid) was composed of 3 levels (1- and 5-mM concentrations) and a control (distilled water). The experimental design was a complete randomized design with three replications. For biochemical data, the protein content and trypsin inhibitory activity of the protein fractions were analyzed under a complete randomized design. The analysis was performed using SAS software version 8 (SAS Institute, Cary, North Carolina, USA), and comparisons of means were performed by Tukey's test ($p \leq 0.05$) when ANOVA showed significant differences among treatments.

RESULTS

There were highly significant differences in the effects of germination time and concentration of salicylic acid on the variable's radicle length and percentage of germination, which SA and germination time influenced radicle length and percentage of germination. The interaction effect of both factors on radicle length was significantly high, which indicates that the germination response depends on the concentration of SA. No significant interaction was detected between the factors evaluated for the percentage of germination.

No significant differences were observed in the radicle length (Figure 1) among the treatments applied during the first 24 h of the test (day 1). However, from day two onward, a significant difference was observed in the 5 mM SA treatment, with a value of 13 mm compared to the control and 1 mM SA treatments, with values of 21.6 and 19.6 mm, respectively. After 72 h (day 3), the length of the radicle of the induced seedlings was delayed the growth by 20 and 60% in the 1 mM SA and 5 mM SA treatment groups, respectively, compared to that in the control group. At the end of the experiment (96 h), the length of the radicle in both treatments with SA remained constant with respect to the previous measurement (72 h), while the length of the radicle in the control treatment continued normal development. These results indicate that SA has a negative effect on the development of the radicle, and the effect is dependent on the concentration of SA.

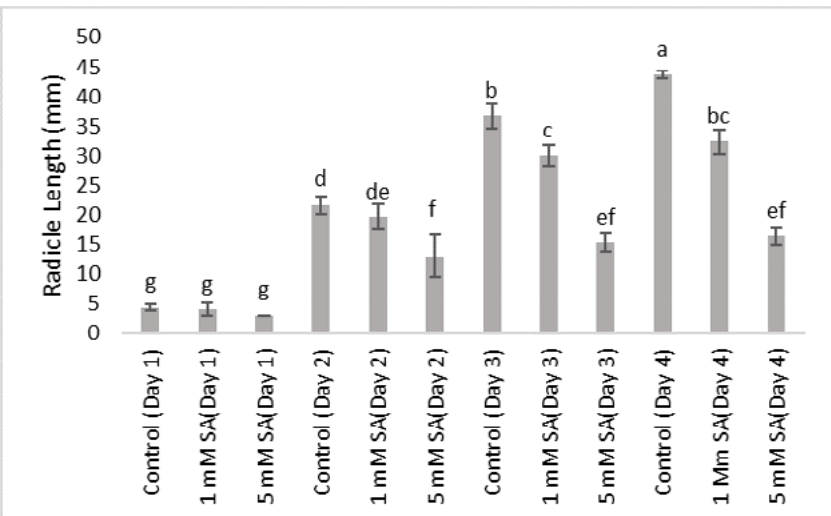

Figure 1. Radicle length of chickpea seedlings chemically induced with SA. The data are expressed as the means (Tukey's test, $p \leq 0.05$). Day 1 (24 h); Day 2 (48 h); Day 3 (72 h); Day 4 (96 h); Control: seeds germinated with distilled water; SA: Salicylic acid.

In Figure 2 can be observed the interaction of treatments and time. From day 1, there was a significant difference in the treatment with 5 mM SA compared to the control (17% lower) and with 1 mM SA (21% lower). However, on the last day of the test (day 4), the germination percentages were similar among the treatments.

Regarding the speed of germination (Figure 3A), no effect of SA was observed; the seeds treated with both concentrations of SA (1 mM and 5 mM) had a speed of germination similar to that of the control. For the root growth rate (Figure 3B), a difference of 28.3% and 68% less in size was observed for the 1 mM SA and 5 mM SA treatments, respectively, compared to the control.

The protein content of the fractions showed that the albumin fraction was approximately 50% lower in the seedlings than in the non-germinated seeds. It was also observed that among the seedlings induced with SA, the 5 mM SA treatment had a greater protein content (32%) compared with the 1 mM SA treatment (Figure 4A).

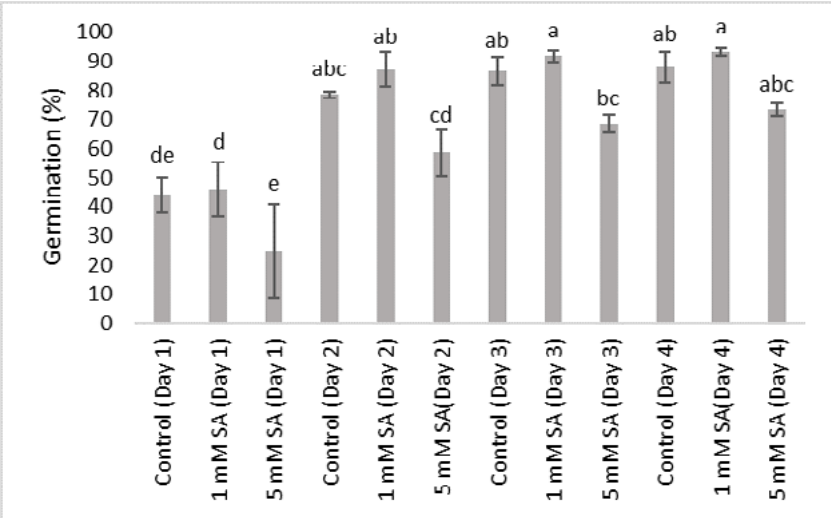

Figure 2. Percentage of germination of chickpea seeds induced with salicylic acid for four days after the onset of the test. The data are expressed as the means (Tukey's test, p≤0.05). Day 1 (24 h); Day 2 (48 h); Day 3 (72 h); Day 4 (96 h); Control: seeds germinated with distilled water; SA: Salicylic acid.

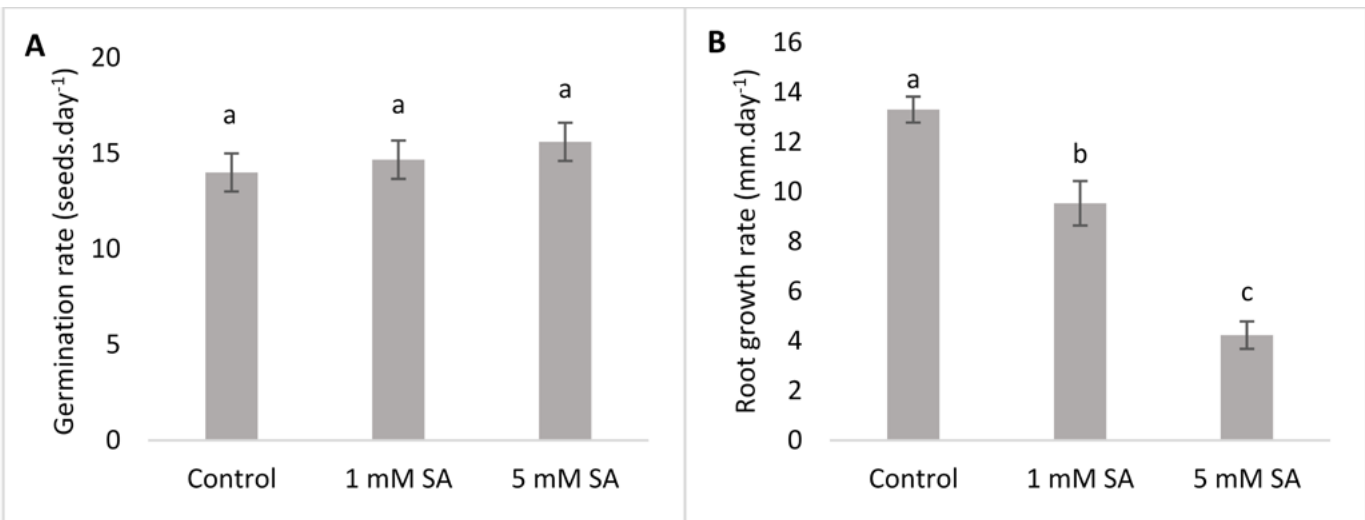

Figure 3. Germination (A) and root growth rates (B) of chickpea seeds chemically induced with SA. The data are expressed as the means (Tukey's test, p≤0.05). Control: seeds germinated with distilled water.

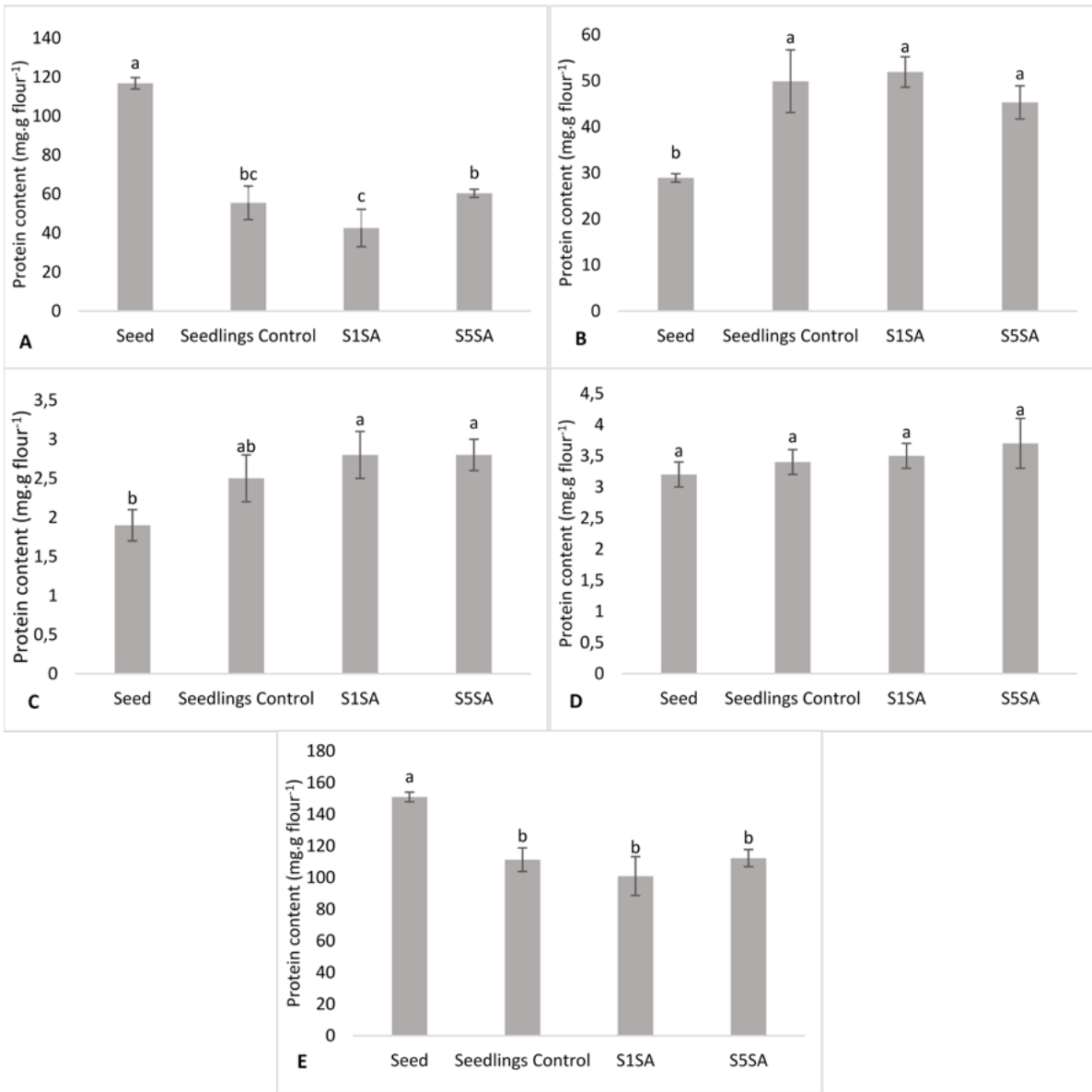

Figure 4. Protein content of the different fractions of chickpea seeds and seedlings. The data are expressed as the means. S1SA, Seedlings 1 mM SA-induced. S5SA, Seedlings 5 mM SA-induced. A, Albumin. B, Globulin. C, Prolamin. D, Glutelin, and E, Total protein. Different letters in each graph indicate statistically significant differences (Tukey's test, $p \leq 0.05$). SA: salicylic acid.

Regarding the globulin fraction, the seedlings protein content increased by more than 70% compared with that in the non-germinated seeds, but no difference was detected in the protein content of this fraction between the seedlings induced with SA and those not induced. Concerning the prolamin fraction (Figure 4C), no differences were observed in the protein content between the seedlings induced with SA and those not induced, nor was a change observed in the protein concentration of this fraction between the non-germinated seed and the seedlings from non-induced seeds. However, an average of 45% increase in the protein concentration of this fraction was observed in the seedlings induced with SA compared to the non-germinated seeds. The protein concentration of the glutelin fraction did not change in any of the seedlings (Figure 4D), either induced with SA or not induced, nor did this protein fraction of the seedlings change in comparison with that of the non-germinated seeds. Finally, the total soluble protein content differed by 28% between the non-germinated seeds and the SA-treated seedlings (1 mM SA, 5 mM SA and seedlings control without induction) (Figure

4E). Nevertheless, no significant differences in total soluble protein content were detected between the seedlings induced with SA (1 mM and 5 mM) and the seedlings without chemical induction (seedling control) (Figure 4E).

The protein fraction results showed that the amount of TIA in the albumin solution was not affected by the effect of germination or by the effect of induction with SA during the germination process (Figure 5A). However, the TIA in the globulin fraction was 77 and 31.6% lower in the seedlings from the 1 mM SA and 5 mM SA treatments, respectively, compared to non-germinated seeds, and 78.3 and 34.8% lower in the seedlings from 1 mM SA and 5 mM SA treatments, respectively, compared to the seedlings from the control (with no SA induction) (Figure 5B).

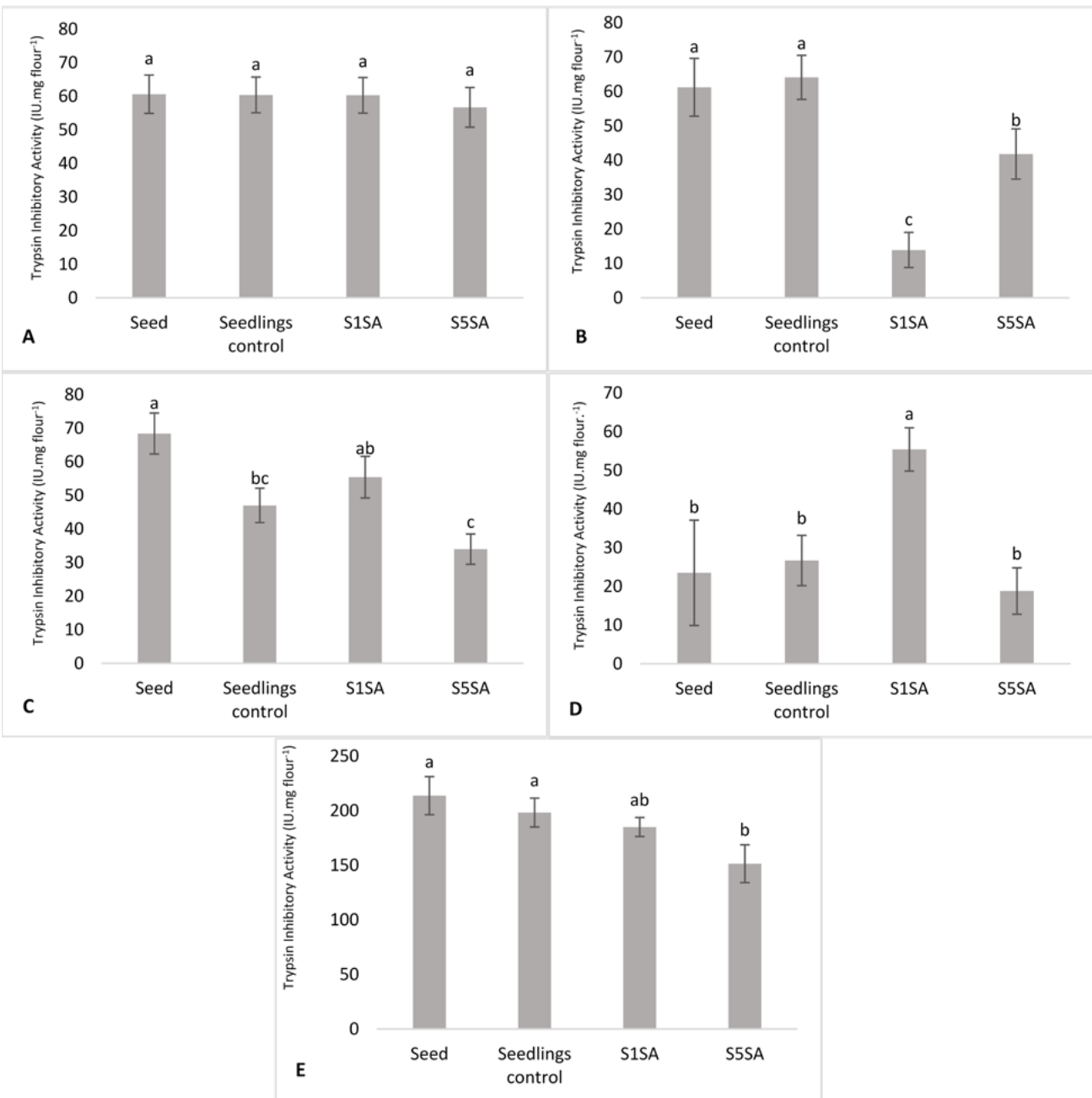

Figure 5. Trypsin inhibitory activity in chickpea seed and seedlings protein fractions. The data are expressed as the means. S1SA, Seedlings 1 mM SA-induced. S5SA, Seedlings 5 mM SA-induced. A, Albumin. B, Globulin. C, Prolamin. D, Glutelin, and E, Total protein. Different letters in each graph indicate statistically significant differences (Tukey's test, $p \leq 0.05$). SA: salicylic acid.

In the case of the TIA in the prolamin fraction, a decrease of 31% was observed in the seedlings from the control compared to those from the non-germinated seeds, however, when the TIA of the control seedlings was compared with that of the seedlings induced with SA, there were no differences. Nevertheless, when comparing the TIA of this protein fraction between the seedlings induced with SA, the 5 mM SA treatment group was 30% lower than the 1 mM SA treatment group (Figure 5C). The glutelin fraction in the TIA of the seedlings treated with 1 mM SA was two and three times greater than that in the control seedlings and the seedlings treated with 5 mM SA, respectively (Figure 5D).

The total trypsin inhibitory activity (TIA) was not significantly modified during germination or by induction with 1 mM SA; however, it was reduced by 33% in the seedlings induced with the 5 mM SA treatment compared to the control seedlings (Figure 5E).

The changes in the protein concentrations of the different fractions can be identified using polyacrylamide gel electrophoresis (PAGE). In Figure 6A can be observed the bands of the electrophoretic patterns present in the albumin fractions (lanes 1-4), and in Figure 6B can be observed the bands of the globulin fractions (lanes 1-4). In relation to the albumin fraction, the protein bands at 66 and 50 kDa (black arrow) were degraded during the germination process (lane 2, non-SA-induced germinated); however, in the SA-induced seedlings, these bands were not degraded (lanes 3 and 4). A similar effect was observed for the doublet bands slightly below the 45 kDa marker (yellow arrow), which the intensity of the doublet bands was significantly weaker in the non-induced seedlings (densitometric value, 110.7), with 15.2% and 6.7% less intensity, than in the seedlings induced with 5 mM (densitometric value, 131.3) and 1 mM SA (densitometric value, 119.3), respectively.

The globulin fraction showed similar protein patterns in each treatment, where no difference in protein degradation was observed in this fraction due to the effect of germination or the effect of SA treatment on the germination process. In contrast, the bands with molecular weights between 40 and 45 kDa (black arrow) were significantly more intense, with 12% and 10% in the seedlings induced with SA (densitometric value, 235.3) (Figure 6B, lanes 3 and 4) and in the seedlings from non-induced seeds (densitometric value, 230.7) (lane 2), respectively, compared to the non-germinated seeds (densitometric value, 209.5) (Figure 6B, lane 1).

In the prolamin fraction (Figure 7A), few differences in protein pattern were observed; a 55 kDa protein was detected in non-induced seedlings (densitometric value, 142.5) (lane 2, black arrow). However, in the fractions obtained from

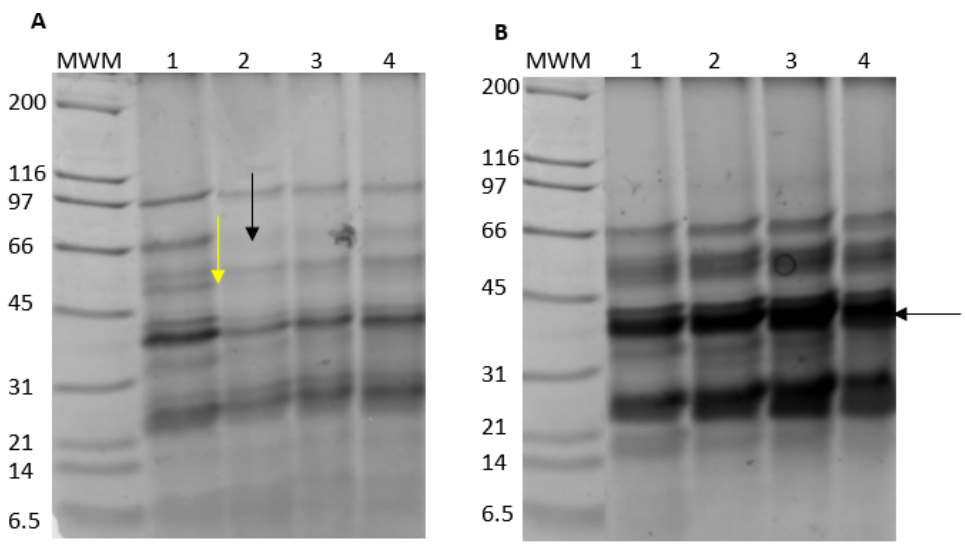

Figure 6. Protein profiles of chickpea seeds and seedlings induced with salicylic acid. (A) Albumin (lanes 1-4) and (B) globulin (lanes 1-4) fractions. where MWM: molecular weight marker (kDa); lane 1: non-germinated seeds; lanes 2: control seedlings with distilled water; lanes 3: seedlings induced with 1 mM salicylic acid; and lanes 4: seedlings induced with 5 mM salicylic acid.

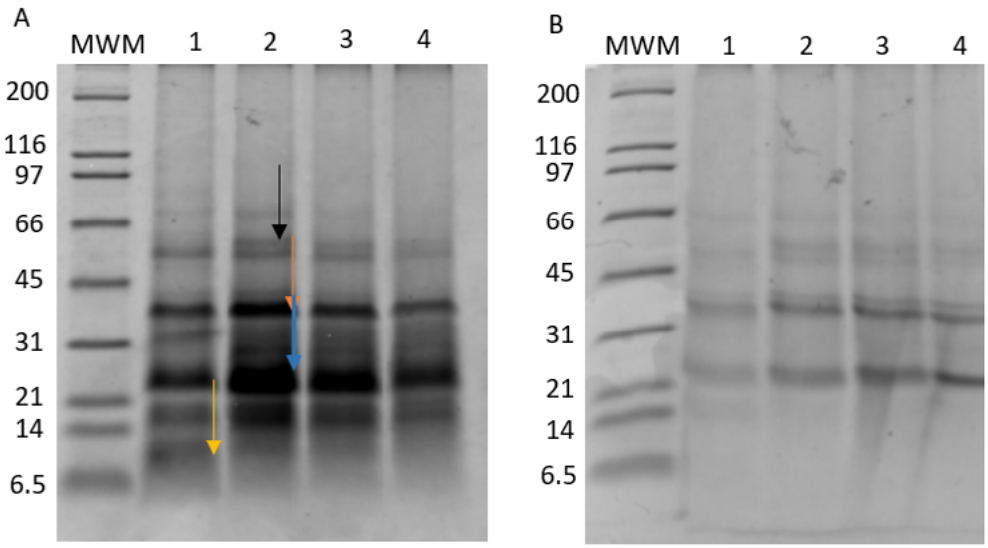

Figure 7. Protein profiles of the prolamin (A) and glutelin (B) fractions of chickpea seeds and seedlings induced with salicylic acid. MWM: Molecular weight marker; 1: Non-germinated seeds; 2: Non-SA-induced control seedlings; 3: Seedlings induced with 1 mM salicylic acid; 4: Seedlings induced with 5 mM salicylic acid.

the SA-induced seedlings (lanes 3 and 4), the same protein was significantly less intense (densitometric values, 129.2 and 118.4) (9% and 17%, respectively) when 1 mM and 5 mM SA were used compared to the non-SA-induced seedlings. This effect of SA concentration was more noticeable for other proteins, such as 37 kDa (orange arrow), whose intensity was significantly lower, at 12% (densitometric value, 185.3) and 23% (densitometric value, 161.1), when 1 mM and 5 mM were used, respectively, compared to non-SA-induced seedlings (densitometric, value 211.4). Similarly, the 23 kDa protein (blue arrow) had a significantly more intense band (densitometric value, 230.5) in non-SA-induced seedlings than in 1 mM and 5 mM SA-induced seedlings, with 4% (densitometric value, 221.7) and 15% (densitometric value, 196.4) less intensity, respectively. In the non-germinated seeds, a band close to 10 kDa (lane 1, yellow arrow) could be observed, which was practically imperceptible in the germinated seedlings, regardless of whether they were induced with SA or not (lanes 2-4). Due to the low content of prolamins present in most legumes, the banding pattern has not been reported in previous research, however, low-molecular-weight proteins could be observed with potential biological studies.

Regarding the glutelin fraction (Figure 7B), the largest number of bands were between 21 and 66 kDa; in this fraction, no differences were observed between the bands of the seedlings regardless of SA induction (lanes 2-4). The difference observed was among the protein patterns of the seedlings (induced or not induced by SA) being significantly more intense (densitometric values 112, 121.7, and 124.6 to no SA induced, 5- and 1-mM SA induced seedlings, respectively) with an average of 18% (lanes 2-4), compared to the 24 kDa protein band (densitometric value, 106.2) of the glutelin protein profile of the non-germinated seeds (lane 1); a similar effect was observed for the 37 kDa protein band.

DISCUSSION

Concentrations of SA in the range of micromolar order produce resistance responses in plants to different adverse factors and can also promote greater seedling development (Hayat et al., 2010; Gayatridevi et al., 2012; Vázquez-Hernández et al., 2019; Ding and Ding, 2020). It has been reported that SA applied at relatively high concentrations (millimolar order) can retard or inhibit germination in seeds of corn (*Zea mays*), barley (*Hordeum vulgare*) and *Arabidopsis thaliana* (Rivas-San Vicente and Plascencia, 2011). However, in *Vicia faba* seeds, germination remained at 80% (Anaya et al., 2018), which is similar to what was observed during 'Desi' chickpea germination in this study. When

these concentrations of SA are applied (1 and 5 mM SA), they activate defense metabolite synthesis pathways (Rai et al., 2021) but do not promote seedling development, as was observed in radicle development (Figure 1).

The results indicate that SA has a retardant effect on germination that depends on the concentration of the compound. In this investigation, the concentration of 5 mM SA caused a negative effect on the percentage of germination of chickpea seeds, unlike the concentration of 1 mM. The higher the concentration of the compound, the higher the production of reactive oxygen species (ROS) (Gao et al., 2021), consequently reducing the development of the radicle in the seedlings.

Although it was reported that SA concentrations on the order of millimolar can inhibit germination, in chickpeas, the 1 mM SA concentration only delayed radicle development (compared to the control treatment) but did not inhibit the protrusion of the seed. On the other hand, the concentration of 5 mM SA (5x) not only affected seedling development but also significantly reduced germination. Therefore, the dose-response effect of SA has a small difference in the range of concentrations to be used (Anaya et al., 2018; Gao et al., 2021; Rai et al., 2021).

The changes in the protein concentration of the different fractions due to the application of exogenous SA during germination suggest that it is occurring the hydrolysis of seed storage proteins and/or synthesis of new proteins from the embryonic tissue of the seedlings or by the synthesis of proteins or enzymes that activate secondary metabolism caused by chemical induction.

In the case of albumins, protein bands with similar weights to those of lipoxygenase (87 kDa) and convicillin (62 kDa) were observed in this study, as previously reported for 'Kabuli' chickpea (Serrano-Sandoval et al., 2019). The bands related to convicillin showed a lower intensity during germination, but the intensity of the bands was maintained in the seedlings induced with SA without degradation. Similarly, salt stress studies conducted on two types of chickpea seedlings (susceptible and tolerant to salt stress) showed that both types had similar electrophoretic patterns even after 20 days of stress (Kumar et al., 2020).

The content of globulins in the chickpea seeds ('Desi' type) of this study also showed similar weights to the α subunit of legumin (44 kDa) and to different subunits of vicilins (25-37 kDa) present in 'Kabuli' chickpea (Serrano-Sandoval et al., 2019). Although the changes in the electrophoretic patterns were minimal, hydrolysis of some storage proteins could be observed. The application of SA did not promote the synthesis of new proteins; however, its application affected the concentration of proteins during germination, resulting in lower growth in the radicle of the seedlings (Figure 1).

Chemical induction can be useful in improving seed germination of important crops, which can result in higher crop productivity and yield. However, depending on the concentration applied, it can also have a positive impact on the nutritional and nutritional quality of the sprouts, for example, the reduction of anti-nutritional compounds, or the increase of nutrients such as proteins (Khan et al., 2023).

CONCLUSIONS

The application of high concentrations of SA affects the germination percentage of 'Desi' type chickpea. However, there is a dose/response effect on seedling development due to lower root growth with a higher dose of SA.

Germination and the application of exogenous SA promote changes in the content of the different protein fractions, including a decrease in the albumin content and an increase in the globulin and prolamin contents.

Trypsin inhibitory activity is considerably reduced during germination, where the application of 1 mM SA promotes a reduction in trypsin inhibitory activity in the globulin and prolamin fractions.

The electrophoretic patterns of the protein fractions in general do not show major differences, but hydrolysis of storage proteins during germination (albumins) occurs, as well as the accumulation of globulins and prolamins.

The application of these two different concentrations of SA inhibits the hydrolysis of storage proteins that could have been used for the development of the seedling. Proteins that are not hydrolyzed can be used as a part of the defense system instead of for seedling development.

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